REVIEW

Circadian time signatures of fitness and disease

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Biological clocks are autonomous anticipatory oscillators that play a critical role in the organization and information processing from genome to whole organisms. Transformative advances into the clock system have opened insight into fundamental mechanisms through which clocks program energy transfer from sunlight into organic matter and potential energy, in addition to cell development and genotoxic stress response. The identification of clocks in nearly every single cell of the body raises questions as to how this gives rise to rhythmic physiology in multicellular organisms and how environmental signals entrain clocks to geophysical time. Here, we consider advances in understanding how regulatory networks emergent in clocks give rise to cell type–specific functions within tissues to affect homeostasis.

Developments in genetics and biochemistry have revealed pervasive regulation of cell and organismal function on a 24-hour circadian time scale across all photosensitive forms of life. The observation that photosensitive forms of life exhibit intrinsic timekeeping mechanisms was first established in the 18th century by de Mairan's observation of the autonomous leaf movement of the *Mimosa* plant. Internal circadian clocks emerged in cyanobacteria, the first organism capable of oxygenic photosynthesis, more than 2.5 billion years ago and enabled the anticipation of daily changes in the light-dark environment tied to the rotation of Earth. Insight into the selective advantage of temporal organization in lower organisms has focused on the integral role of clocks in DNA damage repair, the timing of oxygenic photosynthesis, and how temporal organization provides a means of averting futile energetic cycles.

The subsequent discovery of the molecular clock in animals established that genes control behavior. The circadian system is fundamentally a genetically encoded anticipatory mechanism that underlies both gene-environment and brain-behavioral interactions. As such, the study of molecular clocks provides insight into the dynamic control of genome biology and its link to systems physiology (Fig. 1). Here, we review progress in establishing the temporal principles of circadian regulation at the level of genome dynamics, and the role of both proper timing and mistiming in fitness and pathologic conditions. Our focus is mainly on findings applicable to mammalian cell and organismal physiology, although where appropriate, we provide reference to advances from prokaryotic, plant, and *Drosphila melanogaster* experimentation.

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Properties of the core clock and its output mechanisms

Nearly two centuries after de Mairan, Konopka and Benzer demonstrated in *D. melanogaster* that a single locus controls these rhythms in animals, although the molecular and cellular basis for this observation was not known (*1*). Formulation of the properties of these oscillators was transformed more than 14 years later with positional cloning of core clock genes and recognition that these encode transcription factors (TFs) that underlie daily rhythms of locomotor activity.

The discovery that PER (for "Period"), the first identified clock TF, itself undergoes 24 hours cycling (*2*) led to the idea that oscillation of the molecular clock relies on negative feedback, a central tenet in the field. PER is itself a repressor of the expression of transcriptional activators (a CLOCK/BMAL1 or CLOCK/NPAS2 heterodimer in mammals) that function in the forward limb as the transcriptional feedback loop. In mammals, the repressive TFs of the clock include the PER heterodimer partner called CRY (for "Cryptochrome"), in addition to a second level of repression mediated by another TF called Rev-erb (Fig. 2). Each day, entrainment of the clock to light occurs through activation of *Per* gene transcription in the negative limb, followed by its nuclear translocation and then repression of the activator limb (*3*).

All of these factors have been validated by using loss-of-function genetic models in mice. Intriguingly, several clock TFs—including PER, CLOCK, BMAL1, and NPAS2—contain a motif called the PAS domain that is found in other TFs that respond to environmental metabolites, xenobiotics, and oxygen fluctuation. Moreover, Rev-erb is a member of the nuclear receptor (NR) superfamily composed of TFs that respond to hormones, metabolites, and xenobiotics. Indeed, both Rev-erbα and Rev-erbβ have been shown to bind and respond to molecular heme (*4*, *5*), and the activating NR that opposes Rev-erb function, called ROR, is also regulated by metabolites, including cholesterol and other sterols (*6*). Thus, whereas the clock transcriptional process exhibits fixed 24-hour periodicity, the core clock components may also sense fluctuations in the cellular environment (*7*).

In addition to regulation of gene transcription through feedback repression, posttranslational signaling steps plays a central role in setting the correct pace of the clock cycle (Fig. 2). Best characterized in animal clocks has been the action of the casein kinase proteins, which phosphorylate and thereby modulate stability of the PER proteins and temperature invariance of the

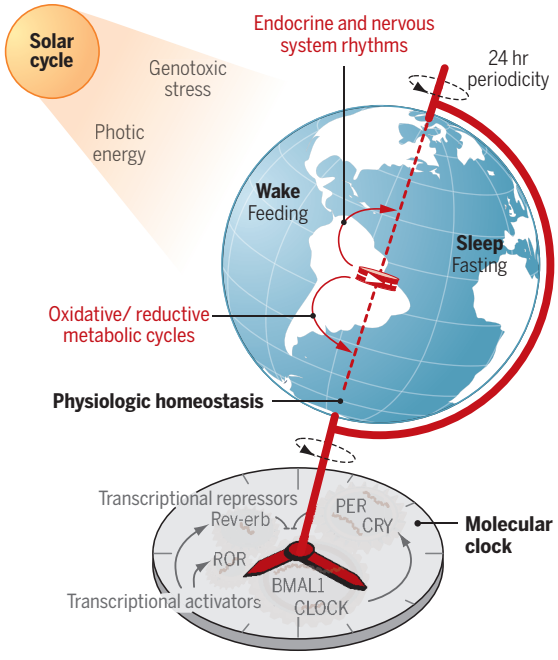

Fig. 1. Geophysical time drives circadian maintenance of homeostasis. The molecular clock is composed of an autoregulatory negative transcription feedback loop that synchronizes physiology and behavior in anticipation of the light-dark cycle. The illustration depicts variation in physiology for diurnal species (active in light); however, circadian cycles also govern sleep/wake and physiologic processes in nocturnal species (active in the dark), with an inverted phase. Exposure to sunlight induces DNA damage each day while also providing energy for oxygenic photosynthesis, processes that may explain the evolution of circadian clocks across four kingdoms of life. Clocks partition oxygenic and reductive metabolic cycles each day and separate these in coordination with the sleep/wake cycle. Although many physiologic processes maintain constancy of the internal milieu, including glucose metabolism, response to perturbation is tuned to circadian time.

clock cycle (*8*). In addition, members of the F-box cullin-like family of ubiquitin ligases Fbxl3 and Fbxl21 mediate CRY stability and proteasomal degradation (*3*). Phenotype-driven genetic screens and proteomic analysis of clock repressor complexes have highlighted the importance of phosphorylation and the interplay between activator and repressor complexes as a determinant of the circadian periodicity (*9*). Despite these advances, fundamental biochemical properties of the circadian clock remain unexplained, especially how the time constant remains invariant even when temperature shifts up to 10°C. Studies in syndromic sleep disorders in which families with heritable very early [familial advanced sleep-phase syndrome (FASPS)] or very late [delayed sleep-phase syndrome (DSPS)] bedtime tendency have identified mutations in the orthologous human clock genes, demonstrating conservation of the central pacemaker molecules across humans (*10*).

Cell type–specific regulation of the core clock and its outputs

The discovery that the transcriptional clock mechanism exists in essentially every cell of the body was a major breakthrough that also raised questions of hierarchy and specificity (*11*). The clock mechanism is composed of TFs that work in the genome by binding to specific DNA sequences; as such, the basic mechanism of transcriptional stimulation and negative feedback is universal. TFs regulate each other's expression via action at enhancer and repressor sites, for which access requires open chromatin. In this context, there is evidence that the core TFs can act as pioneer factors to open chromatin that might otherwise be inaccessible (*12*, *13*).

Yet an early hallmark of the tissue-specific clock transcriptome was the finding that despite the large number of oscillating RNAs in most organs (by some estimates more than 10%) (*14*), the identity of oscillating RNAs is divergent across tissues (*15*). Thus, the distinct clock output of each cell type not only contributes to tissue specificity but to organ-specific manifestations of circadian misalignment (Fig. 3). In addition to controlling each other's expression, core clock TFs bind and directly regulate clock output genes. Many of these clock-controlled genes are expressed in a tissue-specific manner and must therefore be regulated differently than the ubiquitous clock mechanism. An emerging theme in understanding clock-dependent, cell type–specific gene regulation is that core clock TFs act at tissue-specific enhancers established at clock output genes early in development (*16*–*19*).

Critical to this are lineage-specific TFs, which either function as pioneer factors early in ontogeny or bind to the genome at particular regions of chromatin that are already open in specific tissues. Cell type–specific TFs, such as FOXa in liver (*20*) and the pancreatic and duodenal homeobox 1 factor in pancreas (*17*), then contribute to further opening of chromatin through epigenomic mechanisms involving recruitment of transcriptional coregulators, including enzymes that modify histones or

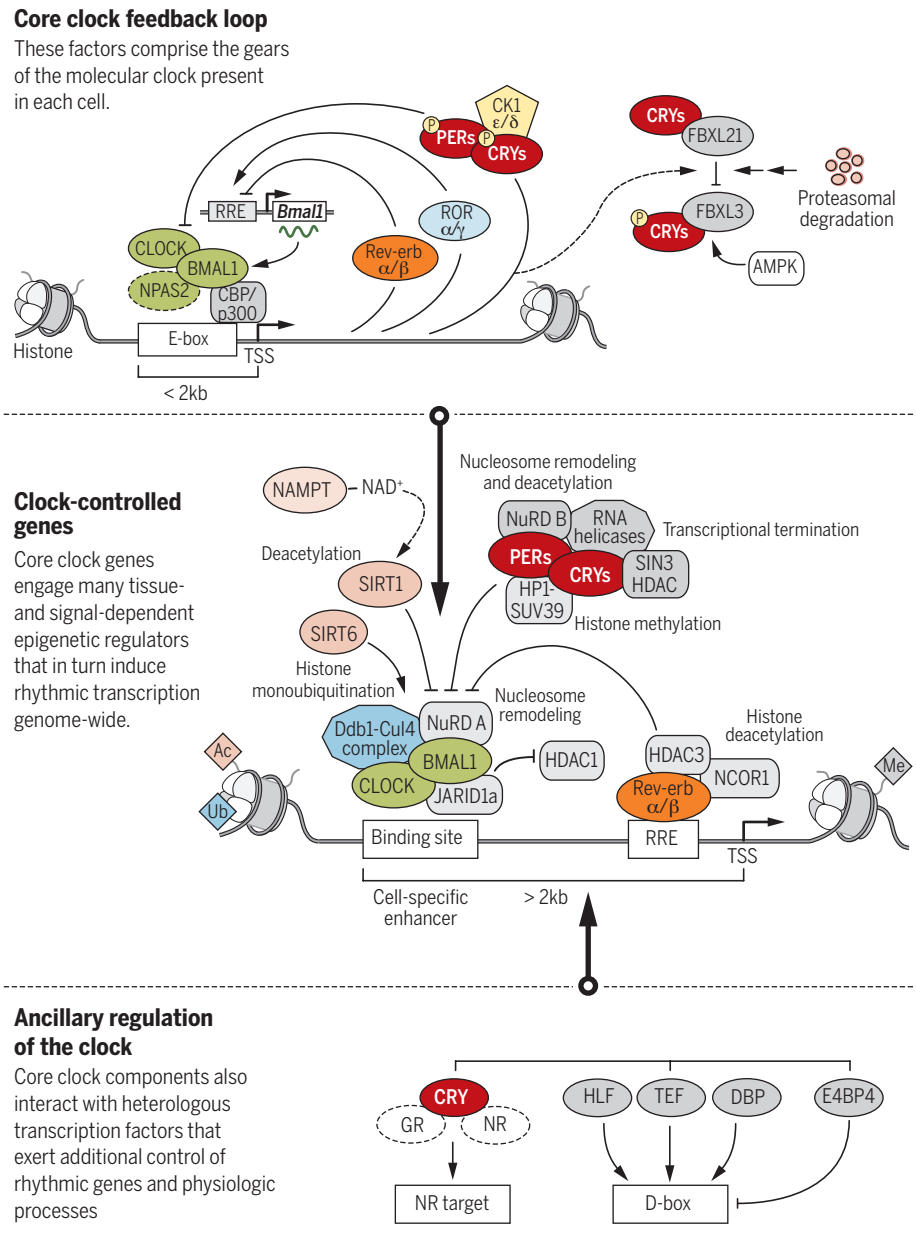

Fig. 2. Molecular regulation of cellular circadian processes. A unifying model of the circadian system involves a defined set of core clock genes that are essential for generation of ~24-hour oscillation in genome-wide transcription. The heterodimeric basic helix-loop-helix TFs CLOCK/BMAL1 and the CLOCK paralog NPAS2 compose the forward limb of the mammalian clock and bind to genomic enhancer elements to positively control circadian clock output genes as well as two distinct repressive pathways required for the negative feedback inherent in clock function. The repressive pathways involve the heterodimeric basic helix-loop-helix TFs PER/CRY and NRs Rev-erbα/β, which function both competitively and noncompetitively with activating ROR NRs at Rev-erb/ROR-response element (RRE)–containing enhancers that control *Bmal1* transcription as well as circadian clock output genes. The clock cycle is regulated through turnover of the repressors after phosophorylation mediated by CK1e/d and executed through ubiquitin-mediated proteasomal degradation involving FBXL3. Posttranscriptional regulation also plays a role in physiologic rhythms, including rhythmic regulation of RNA polyadenylation. Clock factors act through both direct and indirect mechanisms through binding to cell type–specific enhancers far from the transcription start site to regulate a wide array of clock-controlled genes. Both generation of the core clock transcription cycle and its output rhythms engage numerous epigenetic modifiers such as HDACs, methyltransferases, and nucleosome remodeling factors. Clock cycles are also sensitive to environmental signals, including metabolites, DNA damage activation, and signal transduction pathways, all of which feedback to modulate rhythmic transcription but do so differently in distinct tissues in both physiologic and pathologic states. HLF, hepatic leukemia factor; TEF, thyrotroph embryonic factor; DBP, albumin D-box binding protein.

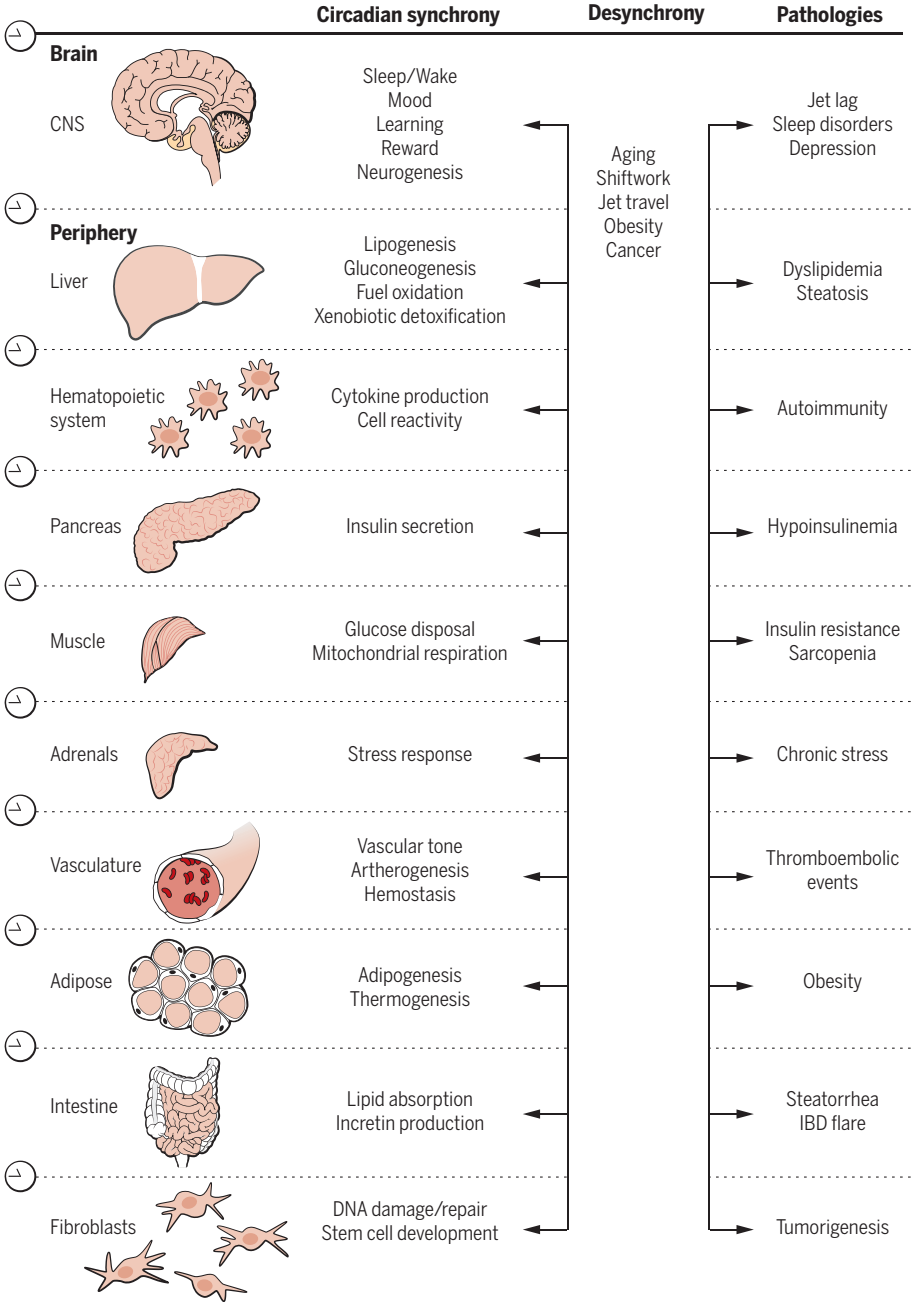

Fig. 3. Circadian systems in physiological cross-talk and disease. The circadian system is organized hierarchically with master pacemaker neurons in the central nervous system entrained to light each day, in turn conducting a distributed network of local clocks expressed in most peripheral cells and tissues. Within the brain, the clock plays a role not only in maintaining the timing of sleep/wake cycle relative to light but also in many behaviors, including learning, reward, and neurogenesis. Peripheral tissue clocks are entrained to the brain clock, although feeding and temperature are dominant in some physiological settings. Peripheral clocks may also become uncoupled and desynchronized from the central pacemaker during aging, shiftwork, jet travel, overnutrition, obesity, or cancer. Circadian disruption and associated impairment in sleep contributes to the molecular pathogenesis of disorders such as metabolic syndrome, obesity, diabetes, autoimmunity, and cancer.

remodel nucleosomes. This opens up binding sites that core clock TFs directly recognize via their DNA-binding specificity, and their binding further alters the epigenome via recruitment of coregulatory complexes. For example, histone acetylation controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs) is highly circadian across the genome at tissue-specific enhancers dictated by core TFs within the liver (*18*, *19*). Further, regulation of histone methylation status also contributes to circadian transcription timing (*21*, *22*). Complementing these direct mechanisms, the core TFs can also act indirectly by tethering to the cell type–specific factor even in the absence of a nearby canonical binding site. For Rev-erbα, this provides a means of regulating circadian output genes independently of RORs, which compete for control of core clock genes (*23*). In this context, there is evidence that the core TFs can act as pioneer factors to open chromatin that might otherwise be inaccessible.

Adding another layer of complexity to genomic activity of circadian TFs has been the identification of interactions between the core clock repressor CRY and the glucocorticoid receptor (GR). CRY binding modulates the balance between activating and repressive functions of the GR (*24*), and this coupling is perhaps consistent with the large number of sites that CRY occupies genome-wide. Moreover, whereas original predictions of clock-controlled genes focused on promoter proximal elements, genomic approaches revealed a broader portrait of clock regulation encompassing a far wider range of cell type–specific locations residing many thousands of nucleotides distant from classical regulatory regions (*16*, *25*).

Regulation of transcription by core clock TFs is also controlled by metabolites such as acetyl coenzyme A (CoA), S-adenosyl methionine, and nicotinamide adenine dinucleotide (oxidized form) (NAD^+). For example, the activating TFs, including BMAL1, recruit HATs, including p300 and CBP [cyclic adenosine monophosphate (cAMP) response element–binding protein (CREB)–binding protein], whose activity requires acetyl CoA, which may be rate-limiting. Furthermore, the activity of the clock proteins can be modulated by their own acetylation, as well as their deacetylation controlled by the sirtuin SIRT1, whose activity is regulated by NAD^+ (*26*, *27*). NAD^+ synthesis is in turn regulated in a circadian manner by the core clock through both direct (*28*, *29*) and indirect control of the rate-limiting salvage pathway enzyme nicotinamide phosphoribosyltransferase (NAMPT), a robustly cycling transcript. NAD^+ levels are further modified by reduction to NADH, as determined by energy transfer, which is cell type–specific and additionally regulated by metabolic demands. The circadian production of NAD^+ also controls the activity of mitochondrial SIRT3, which modulates cellular respiration (*30*). Mounting evidence further suggests that both NAD^+ levels and circadian function decline with age, perhaps in part because of the consumption of NAD^+ with poly(ADP-ribose) polymerase (PARP) activity in response to cumulative genotoxic stress. In this context, reduced organismal "fitness" during aging may be attributable in part to age-related decline in activity of NAD-SIRT1, in turn reducing clock function (*31*). The transcriptional activities of the core clock components of the NR family, Rev-erbα/β and RORα/γ, are additionally regulated by heme and cholesterol, respectively, which are also products of cellular oxidative metabolism (the bidirectional signals affecting the molecular clock cycle are illustrated in Fig. 2).

There are many unanswered questions about the role of the cell-autonomous circadian clock and its tissue-specific function and outputs. In this context, it is important to note that RNA rhythms alone do not capture the full spectrum of clock-controlled networks; for instance, both poly-adenylation and oscillation of the proteome have been shown to exhibit rhythmic regulation (*32*, *33*). Further, rhythmic oscillation of the redox state of the antioxidant peroxiredoxin proteins has been demonstrated in organisms ranging from eubacteria to human red blood cells, indicating that metabolic oscillations may maintain an additional level of timekeeping (*34*, *35*). It has been speculated that the other rhythmic processes, such as the cell cycle, may be coupled to the clock mechanism, and indeed, in isolated cells both the circadian clock and the cell cycles may be synchronized through sequential nutrient restriction and stimulation. Nonetheless, whereas the periodicity of cell cycle varies across tissues in response to nutrient conditions and growth factors, a key feature of the circadian clock is its invariant 24-hour rhythmicity, leaving open the question as to how local factors may align these distinct processes.

Systems-level circadian organization

Although the clock mechanism appears to be quite similar across cell types, it is critical to understand the extent and mechanism of coordination with the central clock. The expression of core clock genes in animals is highly synchronized under free-running conditions via the control of the central clock in the suprachiasmatic nucleus (SCN), which exerts its influence through the rhythmic integration of the sleep-wake cycle, neuroendocrine circuits, and autonomic nervous system. Light, the dominant Zeitgeber of behavioral rhythms, activates specialized photoreceptor cells expressing the pigment melanopsin (*36*, *37*) that in turn tracks via retinal hypothalamic projections to the SCN. SCN neurons exhibit photic resetting through the induction of immediate early genes downstream of the salt-inducible kinase 1 and CREB-regulated transcription coactivator 1 (*38*). Redox flux within the SCN has also been shown to modulate excitability of these neurons (*39*). SCN projections in turn entrain hypothalamic areas central to sleep and arousal through projections to the ventrolateral preoptic area (VLPO), lateral hypothalamic area (LHA), and midbrain [addressed in depth in (*40*)].

Although the molecular underpinnings of sleep remain largely mysterious, analyses of narcolepsy, a syndrome characterized by abrupt episodes of somnolence and cataplexy, have uncovered a role of the hypocretin/orexin as a neuropeptide connecting circadian and metabolic systems. Indeed, mutations in the ORX2 receptor lead to a Mendelian form of canine narcolepsy (*41*), whereas in mice, ablation of the ORX receptor alters energetics and susceptibility to diet-induced obesity (*42*). Further support for a functional convergence between circadian systems and energy balance comes from the finding that genetic disruption of the molecular clock in mice leads to a combination of sleep disturbance (FT), obesity, and metabolic syndrome (*43*). Indeed, the observation of sleep alteration across most genetic models of circadian TF disruption underscores the interconnection between circadian and sleep processes (*44*). Further, mutations of mouse and human Dec2, which represses clock activator proteins, also leads to short sleep (*45*). Intriguingly, human subjects with narcolepsy display a tendency for increased body weight, emphasizing the close molecular, genetic, and anatomic interplay between centers controlling sleep-wake cycles, appetitive circuits, and energy balance (*46*).

A distinct aspect of SCN neurons is that the rhythmic output from these cells arises from neuronal coupling, which is thought to render the pacemaker highly stable to signals that shift peripheral tissue clocks (*47*), most notably the liver, which can be entrained by restricting food availability to the light period when animals are not typically eating and at rest (*48*, *49*). In addition to feeding schedule, the nutritional state of an organism can have major effects on circadian rhythms of extra-SCN neurons and peripheral tissues (*50*, *51*), although the detailed mechanisms through which individual tissues respond to such changes remains incompletely known.

The circadian clocks of cultured cells can also be synchronized by glucocorticoids and temperature shifts (*52*–*54*). In addition, circadian control of temperature rhythms in turn generates rhythmic induction of cold-inducible RNA-binding protein, generating rhythmic variation in RNA splicing (*55*). Moreover, there is evidence that the peripheral clocks of intact organisms can be synchronized by an as yet unknown blood-borne factor that activates transcription mediated by the serum responsive factor (*56*). Furthermore, systemic changes in the metabolic state of the organism emanating from pathology in one tissue (for example, hypoxia due to lung disease) could lead to changes in metabolites, such as NAD^+ affecting other tissues and cell types in the manner described above. Also, the systemic burden of cancer can alter circadian rhythms in peripheral tissues, and circadian disruption may exacerbate tumor growth (*57*). A major unanswered question concerns the interplay of these multiple systemic factors in controlling the phase and synchrony of circadian rhythms in the living organism. This is a very complex issue, but progress will be critical to better understand normal physiology as well as the mechanisms by which circadian desynchrony predisposes to disease. Another unresolved point is whether clock TFs might participate in early stages of tissue ontogeny, as suggested by studies of clock function in stem cell differentiation and malignancy (*58*, *59*).

Physiology and pathophysiology: What's right in fitness and goes wrong in disease?

An anchoring concept in considering how and why timing has evolved derives from Bernard and Cannon's concept of homeostasis as the self-organizing ability of multicellular organisms to maintain a constant internal milieu. Whereas homeostasis in a biochemical pathway adjusts to the steady state with environmental perturbation, the circadian oscillatory process enables anticipation of recurrent variation arising from the light-dark cycle. Clocks represent a paradigm of how genes interact with environment and, in animals, how the central nervous system coordinates behavior with peripheral tissue physiology. Organ-specific disease states ensue when these mechanisms are challenged beyond the limits of homeostasis (Fig. 3).

The observation that clocks with near-24-hour periods are found across all kingdoms of life suggests that intrinsic timekeeping provides a selective advantage. Resonance studies have shown that variants with short or long period lengths survive best when the external light cycle matches the internal period length in prokaryotes (*60*). Although proof of selective advantage is harder to come by in mammals, there do appear to be health benefits related to circadian alignment. For example, clinical studies in which circadian misalignment has been introduced in a controlled laboratory setting in human subjects result in severe dysregulation of glucose homeostasis, insulin action, and appetitive control (*61*, *62*). Whereas misalignment of feeding time with endogenous clock time contributes to diet-induced obesity in mouse models (*50*, *63*), alignment of feeding and activity through restriction of food access to the nighttime in rodents protects from fatty liver (*64*), and similar strategies to control eating time may likewise improve human metabolic health (*65*).

In human subjects, a metric of period length can be estimated based on bedtime and waking propensity because the phase of sleep/wake cycle with respect to day/night hours corresponds with individual chronotype. Such stratification indicates that individuals with mismatched internal circadian cycles with the 24-hour day also exhibit increased risk of disorders such as obesity (*66*). Population analyses such as the Nurse's Health Study further indicate that individuals subjected to shiftwork, a condition characterized by chronic and repeated misalignment between internal clock time and the external light/dark cycle, associated with greater incidence of breast cancer (*67*) and metabolic disease (*68*). Exposure to blue light at night with electronic readers and staying up late on weekends (referred to as social jetlag) may induce circadian disturbance similar to effects of shiftwork (*66*, *69*), suggesting that the risk of shiftwork disorder may be more widespread than anticipated from work schedules alone. Sleep loss itself has been implicated in metabolic and proliferative disorders, and the effects may be difficult to separate from circadian disruption per se because the two processes are intimately interrelated. Although beyond the scope of the present Review, both sleep and circadian disruption have also been tied to neurodegenerative and mood disorders. A unifying concept may be that temporal disruption common in modern society establishes a chronic challenge with respect to circadian

cycles. Our 24-hour clock was fixed in time before the advent of electric light, jet travel, blue light–emitting computer screens, and 24/7 food availability; circadian disruption may be thought of as a sustained environmental stressor leading to ongoing conflict between endogenous biologic cycles and the environment.

Whereas disruption of the circadian system arises under imposed shiftwork conditions, clock function also declines with aging in mammals (*70*), raising the possibility that dysregulation of circadian processes may be a factor contributing to diseases of aging. Indeed, life span extension associated with caloric restriction can be abrogated through disruption of the clock in insects (*71*), whereas disruption of core clock genes has also been associated with hallmarks of early aging in mice (*72*). Insofar as accumulation of DNA damage and cancer risk also increase with aging, emerging epidemiologic and experimental evidence has implicated clock disruption as a factor in tumorigenesis. Studies in Per2 mutant mice have revealed increased radiation-induced lymphoma associated with dysregulation of cell cycle (*73*), whereas abrogation of both activator and repressor arms of the clock was shown to sensitize to lung tumorigenesis (*57*). In contrast, disruption of the *Cry* gene in mice has been implicated in tumor protection because of increased susceptibility to cell death (*74*). Conversely, DNA damage has been shown to shift circadian oscillations because of sequestration of Cry1 by the deubiquitinase Herpes virus–associated ubiquitin-specific protease (*75*). Bidirectional interplay between clock TFs and the oncogene Myc also facilitates tumor cell glycolysis (*76*).

The rhythmic coupling of circadian transcription and DNA repair, involving enzymes such as photolyases, may align the zenith of repair with the peak of ultraviolet genotoxic effects of Sun exposure each day (*77*). Alternatively, circadian cycles involving antioxidant enzymes such as the peroxiredoxins may provide rhythmic protection against the toxic effects of superoxide radicals produced during the oxygenic phase of the photosynthetic reaction cycle. In either case, circadian variation in cell functions has implications for chronopharmacology in cancer and other diseases, in which optimizing treatments may benefit from delivery of agents at specific times in the day/night cycle (*78*).

A key question arising from the connections between circadian function, aging, and organismal health relates to the observation that clocks are present not only in the brain but also throughout the body, where they exert broad effects at every level of organization, from genome regulation to control of protein biosynthesis and cell signaling. In this regard, impaired glucose metabolism is another hallmark of aging that has escalated coincident with the secular trend of circadian disruption due to light at night, shiftwork, and jet travel. In the past decade, experimental and human genetic studies have provided mechanistic insight into cell-specific pathways through which circadian disruption affects disease and metabolic physiology. Intrinsic rhythms of glucose metabolism have been well-characterized in human studies (*79*). Indeed, human genome-wide association studies using blood glucose as a phenotype have identified polymorphisms in both CRY2 (*80*) and the melatonin receptor 1b (MTR1B) (*81*) with glucose levels, which is concordant with experimental genetic analyses showing that circadian gene ablation in pancreas leads to β-cell failure and diabetes mellitus (*82*). Genetic studies have also shown that ablation of the liver clock results in fasting-induced hypoglycemia due to impaired oxidative metabolism, indicating opposing actions of the clock in liver and pancreas. Indeed, the circadian system is crucial for pancreatic β-cell health throughout life because ablation limited to adulthood severely impairs insulin secretion (*17*).

With regard to studies in the mouse, analyses across multiple time points and studies in organotypic explants provide complementary strategies to address whether particular pathologies arise beause of circadian disruption, or instead arise because of actions of clock factors independent of their function in timekeeping. In addition to studies of glucose regulation, clinical and experimental genetic studies have also elucidated the mechanism underlying the clustering of hypertensive crises and myocardial infarction in early morning. Indeed, SCN control of both the autonomic nervous system and neuroendocrine system directly controls daily variation in vascular tone, whereas cell-intrinsic clocks have been shown to contribute to atherogenesis and possibly to thromboembolic disease (*83*, *84*). Uncovering new facets of pathophysiology in individuals with shiftwork disorder such as diabetes mellitus and cardiovascular disease may pave the way to specifically treat the molecular clock in attempts to reverse diseases of misalignment. Although still in an early phase, small-molecule agonists of the clock have been shown to exert beneficial effects on metabolism (*85*).

Conclusions

The mechanism of circadian timing can now be understood as involving negative transcriptional feedback driving self-sustained rhythms within cells and organisms in anticipation of the light-dark cycle. The molecular circadian clock exerts broad effects at each level of organization, from gene transcription to inter-organ communication that is essential for physiologic homeostasis. Although remarkable strides have been made in identifying core components of the clock, major questions remain regarding the biophysical mechanisms underlying transcriptional oscillations, including an elucidation of the kinetic determinants of the 24-hour harmonic, the origin of temperature invariance, and the basis of entrainment. Increasing evidence has revealed an important influence of the clock cycle on genome-wide transcription, although how core clock factors affect the assembly and interplay of coactivator and corepressor complexes to modulate transcription, particularly within cell type–specific enhancers, and how posttranslational modifications, nutrient environment, and cell signaling might influence transcriptional cycles remains incompletely understood. Still unresolved is the question of whether biochemical reactions, such as redox oscillators, might produce autonomous circadian rhythms in anucleated cells or in tissues devoid of transcription.

The societal custom of moving the clock backward each fall provides a reminder of the potential for conflict between artificial light and our inner timepiece, which is entrained by the Sun and set to the 24-hour day. Understanding how regulatory networks emergent in the clock maintain temporal homeostasis has implications for the design of both human and experimental animal studies, in which time of day has been largely ignored. Including time as a variable may open new insight into pathophysiology in basic studies of behavior, metabolism, cardiovascular disease, cancer, and even aging. This concept extends from the organism to its component tissues, whose specialized functions depend on circadian rhythms much as varied genres of music all rely on time signatures. Indeed, the full reach of time was eloquently captured by jazz great Duke Ellington with his declaration that "It don't mean a thing if it ain't got that swing."

REFERENCES AND NOTES

1. R. J. Konopka, S. Benzer, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2112–2116 (1971).
2. P. E. Hardin, J. C. Hall, M. Rosbash, *Nature* **343**, 536–540 (1990).
3. C. L. Partch, C. B. Green, J. S. Takahashi, *Trends Cell Biol.* **24**, 90–99 (2014).
4. L. Yin *et al*., *Science* **318**, 1786–1789 (2007).
5. S. Raghuram *et al*., *Nat. Struct. Mol. Biol.* **14**, 1207–1213 (2007).
6. J. A. Kallen *et al*., *Structure* **10**, 1697–1707 (2002).
7. B. E. McIntosh, J. B. Hogenesch, C. A. Bradfield, *Annu. Rev. Physiol.* **72**, 625–645 (2010).
8. A. Mehra *et al*., *Cell* **137**, 749–760 (2009).
9. J. M. Hurley, J. J. Loros, J. C. Dunlap, *Trends Biochem. Sci.* **41**, 834–846 (2016).
10. C. R. Jones, A. L. Huang, L. J. Ptáček, Y.-H. Fu, *Exp. Neurol.* **243**, 28–33 (2013).
11. A. Balsalobre, F. Damiola, U. Schibler, *Cell* **93**, 929–937 (1998).
12. J. S. Menet, J. Rodriguez, K. C. Abruzzi, M. Rosbash, *eLife* **1**, e00011 (2012).
13. K. S. Zaret, J. Lerner, M. Iwafuchi-Doi, *Mol. Cell* **62**, 665–667 (2016).
14. S. Panda *et al*., *Cell* **109**, 307–320 (2002).
15. K.-F. Storch *et al*., *Nature* **417**, 78–83 (2002).
16. B. Fang *et al*., *Cell* **159**, 1140–1152 (2014).
17. M. Perelis *et al*., *Science* **350**, aac4250 (2015).
18. D. Feng *et al*., *Science* **331**, 1315–1319 (2011).
19. N. Koike *et al*., *Science* **338**, 349–354 (2012).
20. C. S. Lee, J. R. Friedman, J. T. Fulmer, K. H. Kaestner, *Nature* **435**, 944–947 (2005).
21. L. DiTacchio *et al*., *Science* **333**, 1881–1885 (2011).
22. H. A. Duong, C. J. Weitz, *Nat. Struct. Mol. Biol.* **21**, 126–132 (2014).
23. Y. Zhang *et al*., *Science* **348**, 1488–1492 (2015).
24. K. A. Lamia *et al*., *Nature* **480**, 552–556 (2011).
25. G. Rey *et al*., *PLOS Biol.* **9**, e1000595 (2011).
26. G. Asher *et al*., *Cell* **134**, 317–328 (2008).
27. Y. Nakahata *et al*., *Cell* **134**, 329–340 (2008).
28. Y. Nakahata, S. Sahar, G. Astarita, M. Kaluzova, P. Sassone-Corsi, *Science* **324**, 654–657 (2009).
29. K. M. Ramsey *et al*., *Science* **324**, 651–654 (2009).
30. C. B. Peek *et al*., *Science* **342**, 1243417 (2013).
31. H.-C. Chang, L. Guarente, *Cell* **153**, 1448–1460 (2013).
32. S. Kojima, E. L. Sher-Chen, C. B. Green, *Genes Dev.* **26**, 2724–2736 (2012).
33. A. B. Reddy *et al*., *Curr. Biol.* **16**, 1107–1115 (2006).
34. R. S. Edgar *et al*., *Nature* **485**, 459–464 (2012).
35. I. S. Kil *et al*., *Mol. Cell* **59**, 651–663 (2015).
36. I. Provencio, G. Jiang, W. J. De Grip, W. P. Hayes, M. D. Rollag, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 340–345 (1998).

37. S. Hattar *et al.*, *Nature* **424**, 75–81 (2003).
38. A. Jagannath *et al.*, *Cell* **154**, 1100–1111 (2013).
39. T. A. Wang *et al.*, *Science* **337**, 839–842 (2012).
40. C. B. Saper, T. E. Scammell, J. Lu, *Nature* **437**, 1257–1263 (2005).
41. L. Lin *et al.*, *Cell* **98**, 365–376 (1999).
42. H. Funato *et al.*, *Cell Metab.* **9**, 64–76 (2009).
43. F. W. Turek *et al.*, *Science* **308**, 1043–1045 (2005).
44. A. D. Laposky, J. Bass, A. Kohsaka, F. W. Turek, *FEBS Lett.* **582**, 142–151 (2008).
45. Y. He *et al.*, *Science* **325**, 866–870 (2009).
46. A. Aran *et al.*, *Sleep* **33**, 1457–1464 (2010).
47. A. C. Liu *et al.*, *Cell* **129**, 605–616 (2007).
48. F. Damiola *et al.*, *Genes Dev.* **14**, 2950–2961 (2000).
49. K. A. Stokkan, S. Yamazaki, H. Tei, Y. Sakaki, M. Menaker, *Science* **291**, 490–493 (2001).
50. A. Kohsaka *et al.*, *Cell Metab.* **6**, 414–421 (2007).
51. K. L. Eckel-Mahan *et al.*, *Cell* **155**, 1464–1478 (2013).
52. A. Balsalobre *et al.*, *Science* **289**, 2344–2347 (2000).
53. C. Saini, J. Morf, M. Stratmann, P. Gos, U. Schibler, *Genes Dev.* **26**, 567–580 (2012).
54. E. D. Buhr, S.-H. Yoo, J. S. Takahashi, *Science* **330**, 379–385 (2010).
55. I. Gotic *et al.*, *Genes Dev.* **30**, 2005–2017 (2016).
56. A. Gerber *et al.*, *Cell* **152**, 492–503 (2013).
57. T. Papagiannakopoulos *et al.*, *Cell Metab.* **24**, 324–331 (2016).
58. P. Janich *et al.*, *Nature* **480**, 209–214 (2011).
59. R. V. Puram *et al.*, *Cell* **165**, 303–316 (2016).
60. Y. Ouyang, C. R. Andersson, T. Kondo, S. S. Golden, C. H. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 8660–8664 (1998).
61. O. M. Buxton *et al.*, *Sci. Transl. Med.* **4**, 129ra43 (2012).
62. A. W. McHill *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 17302–17307 (2014).
63. D. M. Arble, J. Bass, A. D. Laposky, M. H. Vitaterna, F. W. Turek, *Obesity (Silver Spring)* **17**, 2100–2102 (2009).
64. M. Hatori *et al.*, *Cell Metab.* **15**, 848–860 (2012).
65. D. Jakubowicz, O. Froy, J. Wainstein, M. Boaz, *Steroids* **77**, 323–331 (2012).
66. T. Roenneberg, K. V. Allebrandt, M. Merrow, C. Vetter, *Curr. Biol.* **22**, 939–943 (2012).
67. E. S. Schernhammer *et al.*, *J. Natl. Cancer Inst.* **93**, 1563–1568 (2001).
68. A. Pan, E. S. Schernhammer, Q. Sun, F. B. Hu, *PLOS Med.* **8**, e1001141 (2011).
69. A.-M. Chang, D. Aeschbach, J. F. Duffy, C. A. Czeisler, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 1232–1237 (2015).
70. T. J. Nakamura *et al.*, *J. Neurosci.* **31**, 10201–10205 (2011).
71. S. D. Katewa *et al.*, *Cell Metab.* **23**, 143–154 (2016).
72. R. V. Kondratov, A. A. Kondratova, V. Y. Gorbacheva, O. V. Vykhovanets, M. P. Antoch, *Genes Dev.* **20**, 1868–1873 (2006).
73. L. Fu, H. Pelicano, J. Liu, P. Huang, C. Lee, *Cell* **111**, 41–50 (2002).
74. N. Ozturk, J. H. Lee, S. Gaddameedhi, A. Sancar, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 2841–2846 (2009).
75. S. J. Papp *et al.*, *eLife* **4**, e04883 (2015).
76. B. J. Altman *et al.*, *Cell Metab.* **22**, 1009–1019 (2015).
77. S. Gaddameedhi, C. P. Selby, W. K. Kaufmann, R. C. Smart, A. Sancar, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 18790–18795 (2011).
78. S. Dulong, A. Ballesta, A. Okyar, F. Lévi, *Mol. Cancer Ther.* **14**, 2154–2164 (2015).
79. E. Van Cauter, K. S. Polonsky, A. J. Scheen, *Endocr. Rev.* **18**, 716–738 (1997).
80. J. Dupuis *et al.*, *Nat. Genet.* **42**, 105–116 (2010).
81. N. Bouatia-Naji *et al.*, *Nat. Genet.* **41**, 89–94 (2009).
82. B. Marcheva *et al.*, *Nature* **466**, 627–631 (2010).
83. A. M. Curtis *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 3450–3455 (2007).
84. B. Cheng *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 17147–17152 (2011).
85. Z. Chen, S.-H. Yoo, J. S. Takahashi, *Cell. Mol. Life Sci.* **70**, 2985–2998 (2013).

ACKNOWLEDGMENTS

Work on circadian rhythms is supported in the J.B. laboratory by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) grants R01DK100814 and 2R01DK090625 and in the M.A.L. laboratory by NIDDK grant R01DK45586 and the JPB Foundation. M.A.L. serves on advisory boards for Pfizer and Eli Lilly and Company. J.B. has a financial interest in Reset Therapeutics, a biotechnology company developing therapeutics related to sleep and metabolism. We thank B. Marcheva for the figures. We apologize that space limitations prevented citation of all relevant literature.

10.1126/science.aah4965

REVIEW

Immunity around the clock

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Immunity is a high-cost, high-benefit trait that defends against pathogens and noxious stimuli but whose overactivation can result in immunopathologies and sometimes even death. Because many immune parameters oscillate rhythmically with the time of day, the circadian clock has emerged as an important gatekeeper for reducing immunity-associated costs, which, in turn, enhances organismal fitness. This is mediated by interactions between extrinsic environmental cues and the intrinsic oscillators of immune cells, which together optimize immune responses throughout the circadian cycle. The elucidation of these clock-controlled immunomodulatory mechanisms might uncover new approaches for treating infections and chronic inflammatory diseases.

Virtually all life on Earth is exposed to regular 24-hour environmental cycles generated by the planet's rotation. This in turn has led to the evolution of daily (circadian) rhythms, driven by cell-autonomous biological clocks, which enable organisms to anticipate and adapt to the temporal changes in their environment (*1*). The sleep-wake cycle is perhaps the most obvious output of the circadian system, but numerous other physiological systems are under circadian control, including behavior and locomotor activity; body temperature; the cardiovascular, digestive, and endocrine systems; and metabolic and immune functions (*2*–*7*).

In mammals, the central circadian pacemaker is located in the suprachiasmatic nucleus (SCN), which entrains peripheral clocks found in nearly every cell of the body (*2*, *3*). The SCN oscillator has two distinct properties. First, it is the only part of the circadian system that has retinal innervation, allowing it to be entrained by the solar cycle. Second, unlike the peripheral clocks, which dampen over time, the interneuronal signaling pathways that establish communication between the SCN neurons endow it with an unlimited capacity to generate circadian outputs. At the organism level, circadian coherence in peripheral tissues is maintained by rhythmic generation of entrainment cues by the SCN, including circadian oscillations in body temperature, activity of the sympathetic nervous system (SNS), and circulating concentrations of glucocorticoids. The coherence between central and peripheral circadian clocks confers an adaptive advantage, and its disruption has been suggested to decrease organismal fitness. In support of this, lifestyles that disrupt inherent timing systems, such as exposure to abnormal lighting schedules in chronic shift work, are associated with an increased risk of cancer, metabolic disorders, and cardiovascular and cerebrovascular disease (*4*). Also, many human diseases exhibit circadian rhythmicity in their pathology, including myocardial infarction, asthma, and rheumatoid arthritis (*4*, *5*).

Although diurnal variation in host immune responses to lethal infection was demonstrated over 50 years ago (*8*, *9*), only recently have studies started to uncover the multiple aspects

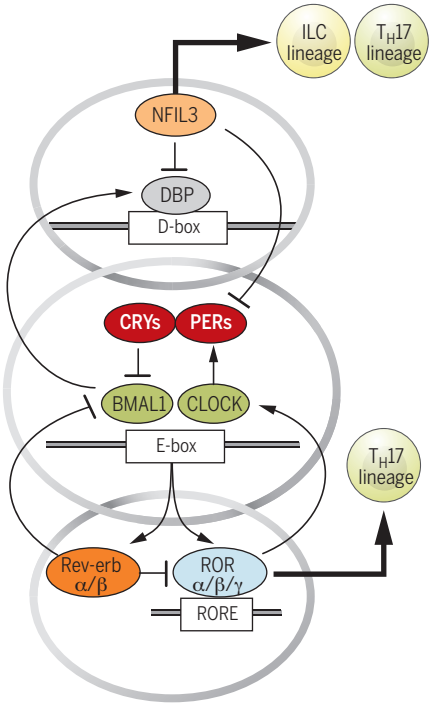

Fig. 1. Interlocking loops of the molecular clock drive immune responses. The circadian pacemaker is controlled by three interlocked transcription-translation feedback loops, involving rhythmic transcriptional repressors that act on D-box, E-box, and RORE sites. Genes driving the core clockwork also regulate multiple noncircadian pathways. Two of the circadian oscillators, NFIL3 and RORα, also regulate development of ILCs and T_H17 cells. Lines terminating in perpendicular bars denote inhibition. DBP, albumin D-box binding protein; CRYs, cryptochromes; PERs, Periods.

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Circadian time signatures of fitness and disease

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Science, 354 (6315), • DOI: 10.1126/science.aah4965